

Sustainable Bacterial Cellulose Production from Avocado Seed Waste Using a Green Biorefinery Approach

Gabriela Barraza-Jáuregui ¹, Yolanda Luciana Abanto Sánchez ¹, Angie Rojas ¹, José Carlos Alcántara ^{1,2,3}, Daniel Antonio Medina Bocanegra ¹, Hernán Alvarado-Quintana ¹, Alberto Flores-Pérez ⁴, Fernando Javier Hurtado Butrón ⁵, Carlos Sopán-Benaute ⁶, María Guadalupe Morán-Aguilar ^{3,*} and Fabiola Vilaseca ^{3,*}

S1. Methods

S1.1. National Renewable Energy Laboratory (NREL) method for lignocellulosic composition determination: carbohydrate quantification

The biomass free of extractives (300±10.0 mg) was subjected to two consecutive sessions of acid hydrolysis. Firstly, 3.00±0.01 mL of 72% H₂SO₄ (w/w) at 1 h and 30°C was applied; then, 84.00±0.04 mL of deionised water was added to dilute the H₂SO₄ to a 4% concentration. The sealed samples were placed in an autoclave for 1h at 121°C. After the autoclave cycle was completed, they were allowed to cool slowly to room temperature (25°C) before the lids were removed. Finally, all the samples were filtered under vacuum, and the supernatant was used for the quantification of acid soluble lignin (ASL) and total structural carbohydrates. The analysis of structural carbohydrates was performed using high-performance liquid chromatography (HPLC) (Thermo Scientific, Ultimate 3000, USA), equipped with a charged aerosol detector or CAD (Thermo Scientific, Corona Veo RS., USA). The chromatographic conditions are shown in **Table S1**. In addition, a kit of sugar reagents, glucose, xylose, galactose, arabinose, and mannose, with a purity of 99.5% from Sigma Aldrich, reaching from 50 to 1000 ug/mL, was used in triplicate to construct the logarithmic calibration curves.

Table S1. HPLC and CAD conditions for structural carbohydrates.

HPLC parameters	Value
Column	Shodex Sugar SP0810 y SP-G-6B
Injection volume	10 µl
Mobile phase	Acetonitrile 11% (v/v) in aqueous phase
Flow rate	0.700 mL/min
Column temperature	80°C
CAD parameters	Value
Detector temperature	50°C
Filter	5 s
Data collection	10 Hz
Execution time	25 min.

Table S2. Fermentation performance parameters measured during bacterial cellulose production.

Medium	Time (days)	Production (g/L)	Productivity (g/L·d)	Consumed RS (%)
HS	5	0.47±0.04	0.009 ± 0.01	66.15±0.48
	10	1.24±0.01	0.012 ± 0.00	79.84±0.11
	15	0.97±0.01	0.006 ± 0.00	95.24±0.17
	20	0.67±0.03	0.003 ± 0.00	95.31±0.09
MHS	5	0.85±0.04	0.017 ± 0.01	71.86±0.44
	10	1.93±0.01	0.019± 0.00	82.08±0.28
	15	1.48±0.04	0.010 ± 0.01	94.59±0.23
	20	1.10±0.03	0.011 ± 0.00	94.70±0.21

HS: Hestrin–Schramm medium; MHS: modified Hestrin–Schramm medium; RS: reducing sugars.

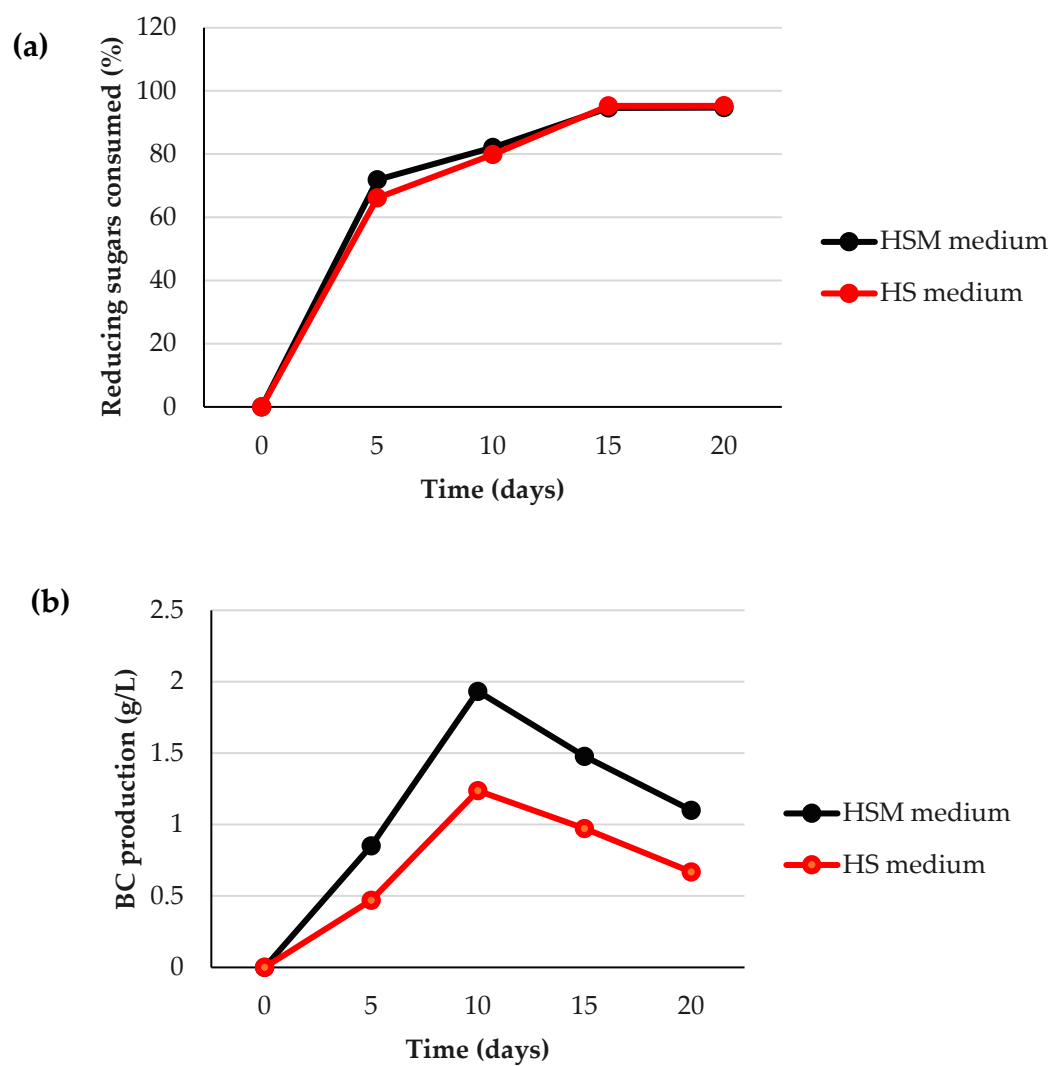


Figure S1. Kinetics of bacterial cellulose production. a) Time-course of reducing sugar consumption during 20-day fermentations in HS and MHS media. b) Corresponding bacterial cellulose productivity profiles.

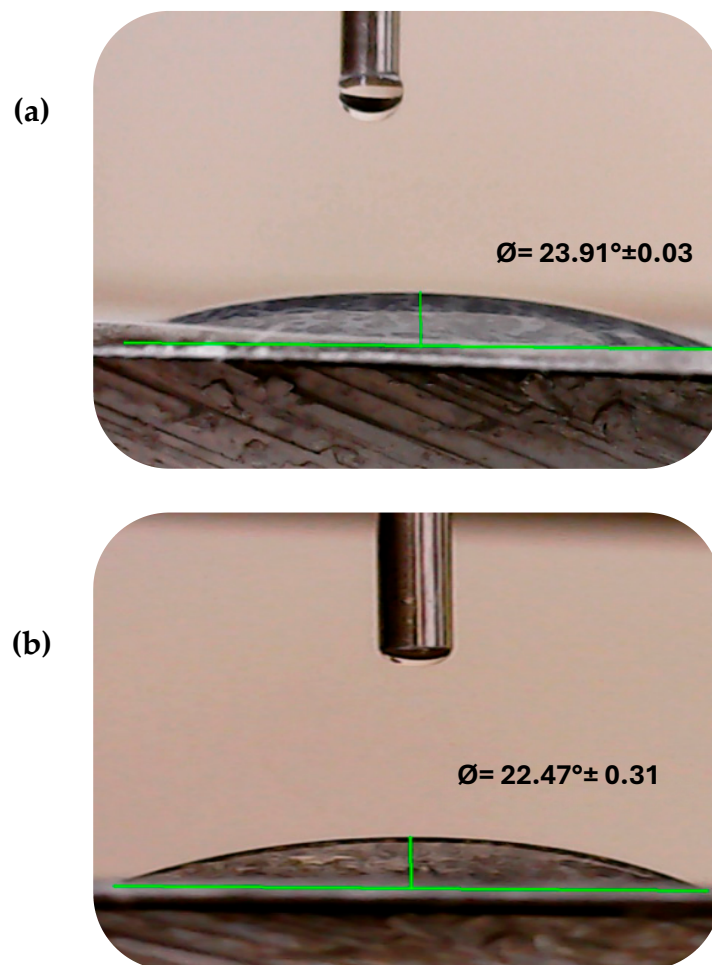


Figure. S2. Static water contact angle values for the BC membrane from a) HS medium and b) MHS medium.